A homozygous nonsense mutation in δ-sarcoglycan exon 3 in a case of LGMD2F

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Abstract

We present the first Turkish family with δ-sarcoglycanopathy (LGMD2F). A novel truncating mutation (E93X) in exon 3 was identified in the gene. The index case showed a severe course and there was no cardiac involvement. LGMD2F seems to be rare in our population. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The autosomal recessive limb girdle muscular dystrophies (LGMD2) are a heterogeneous group of genetic disorders in which there is a progressive weakness of the pelvic and shoulder girdle musculature. Sarcoglycanopathies, a subgroup within LGMD2 are caused by mutations in the genes coding for the four sarcoglycan proteins (γ-sarcoglycan, α-sarcoglycan, β-sarcoglycan and δ-sarcoglycan) [1–7]. In each of these conditions, a mutation in any one sarcoglycan gene results in concomitant deficiency of the entire sarcoglycan complex. Despite rapid advances in clarifying the genetic defects that cause sarcoglycanopathies, little is known about the molecular causes of the disease. Homozygous mutations are particularly valuable for the generation of genotype/phenotype correlation, with respect to sarcoglycan integrity.

In most populations, δ-sarcoglycanopathy is the least common type of sarcoglycanopathy. The δ-sarcoglycan gene was identified by Nigro et al. [6] on the basis of homology to γ-sarcoglycan. The gene was localized to chromosome 5q33–q34, a region to which an autosomal recessive form of LGMD had been mapped independently [5]. A homozygous 1 bp deletion in δ-sarcoglycan was found to segregate with the disease in these families [7]. The gene spans over 100 kb of genomic DNA, contains eight exons and produces a major 8.0 kb mRNA of 3.6 kb. The gene encodes a basic 35 kDa protein of 290 amino acids with a single transmembrane domain [6–7], it is oriented with the N-terminus within the cell (type II transmembrane protein). Biochemically, δ-sarcoglycan was shown to be a member of the sarcoglycan complex [8]. A deletion in the δ-sarcoglycan gene was also found to cause the phenotype of cardiomyopathy with muscular dystrophy in the hamster strain BIO14.6 [9].

Only one homozygous frameshift (deletion of nucleotide 656 in exon 7) [7], two nonsense (R165X, W30X) [10] and one missense (E262K) [11] mutations have been reported to date in all patients with a severe phenotype.

In this report, we present the first family with LGMD2F in the Turkish population caused, by a novel homozygous nonsense mutation associated with a severe clinical course, but without evidence for cardiomyopathy.
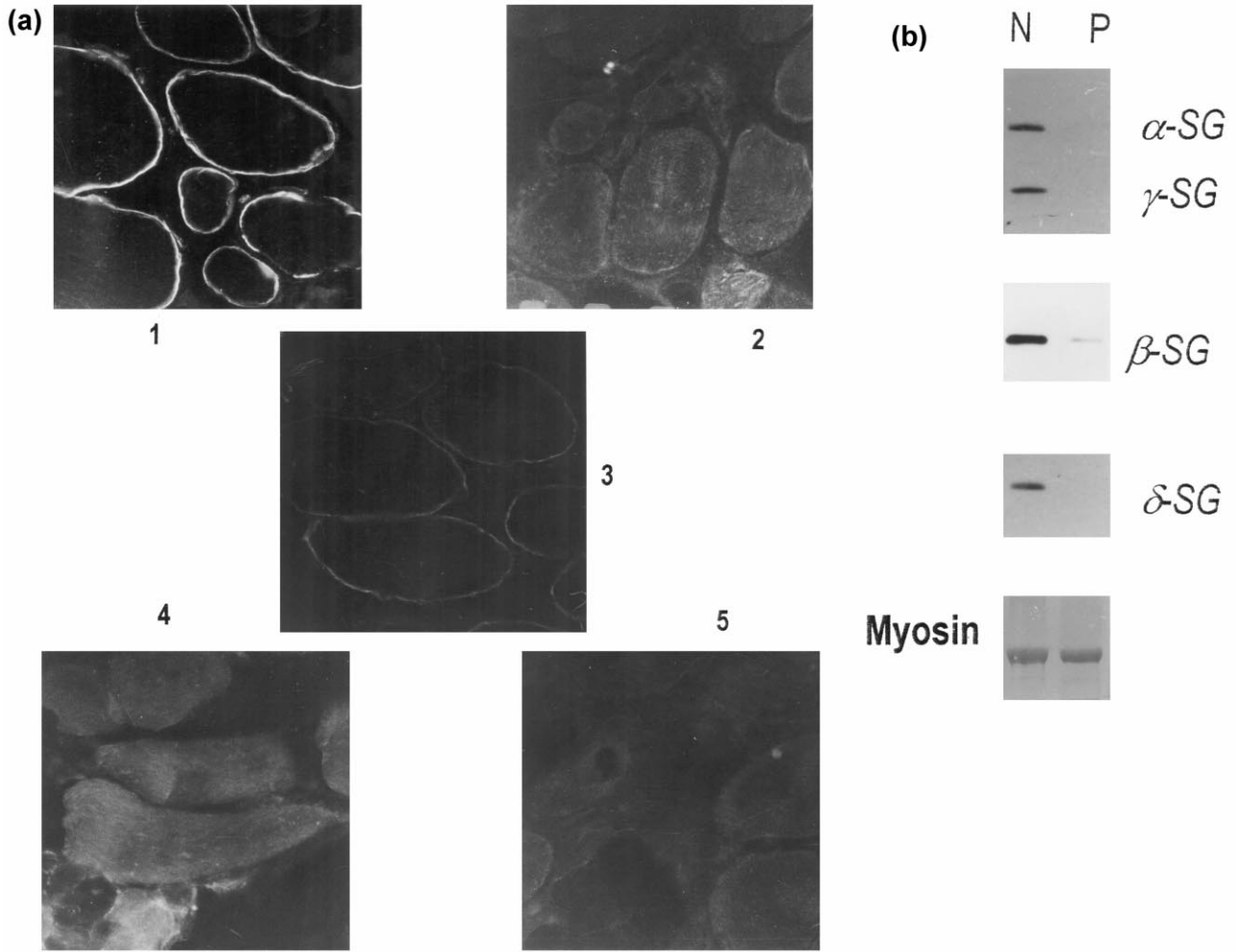
2. Methods

DNA was extracted from peripheral blood samples

Fig. 1. (a) Immunohistochemical analysis of the dystrophin-glycoprotein complex in δ-sarcoglycan-deficient case. (1) Dystrophin (C terminus of the dystrophin); (2) α-sarcoglycan; (3) β-sarcoglycan; (4) γ-sarcoglycan; (5) δ-sarcoglycan. (b) Western blot analysis of α-, β-, γ- and δ-sarcoglycans in δ-sarcoglycan-deficient case. N, normal; P, patient.

according to the standard protocols. Muscle biopsies were processed as described [12] using monoclonal antibodies against dystrophin (NCL-DYS1®, DYS2®, and DYS3®), α-SG (NCL-50DAG®), β-SG (NCL-43DAG®), γ-SG (NCL-35DAG®) and δ-SG (NCL-35DAG®) for immunofluorescence (IF) and Western blot (WB) analysis. For genotyping, highly polymorphic markers of chromosomes 2p13–p16, 4q12, 5q33–q34, 13q12, 15q15.1–q15.3 and 17q12–q21.33 were used in a multiplex system [13]. The mutation was detected by direct sequencing of polymerase chain reaction (PCR) products generated from genomic DNA using primers designed based on Nigro et al. [6].

All studies were performed following patients' informed consent. All sequencing was done on an ABI cycle sequencer.

3. Results

Our index patient is a 16-year-old girl. She is the

offspring to a first degree cousin marriage. Her early developmental milestones were normal and she walked at 12 months of age. Her symptoms started at around 8 years of age with increasing difficulty in climbing stairs and walking. There was a typical proximal muscle weakness with sparing
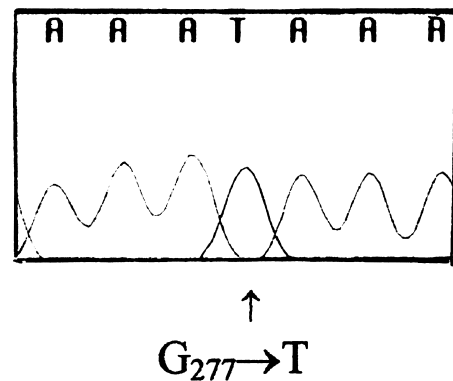

Fig. 2. Mutation data of δ-sarcoglycan deficient case [$G_{277} \rightarrow T$(E93X)].

of the facial and neck muscles. Enlargement of the calves were mildly present in the absence of scapular winging. The evolution was severe; she became wheelchair-bound by age 12. Her CK levels were elevated ×60 and ×55 at ages 9 and 11, respectively. A muscle biopsy at age 11 showed an extensive and active dystrophic process with areas of regeneration, as well. Immunohistochemistry following a routine biotin–avidin method and Western blotting detected virtual absence of α-, γ- and δ-sarcoglycans and severe reduction of β-sarcoglycan in the presence of spectrin, dystrophins and laminin alpha 2 chain (Fig. 1). From age 11 years, her cardiac status was evaluated by EKG and cardiac echogram on a twice yearly basis. No cardiomyopathy has been found to be developed so far. She is a bright young lady and currently receiving physical and occupational therapy. Her pulmonary status has also been stable with vital and forced vital capacity levels being at 76% of the normal.

Genotyping with the markers 336xe9, 329tf5,191xd8-t has compatible linkage to 5q33–q35, as ascertained by homozygosity by descent. δ-Sarcoglycan exons were amplified from genomic DNAs of the patient and her family members and sequenced directly. The propositus was homozygous for a G → T change (G277T), creating a stop codon at amino acid position 93 in δ-sarcoglycan exon 3 Fig. 2)

4. Discussion

Until today, 61 Turkish LGMD2 patients were investigated by protein and DNA data [14,15]. It appeared that, there was a broad spectrum of genes and mutations involved in LGMD2 in Turkey. Almost one third of the patients had sarcoglycan deficiency. In this group, haplotype analysis in only one family was compatible with linkage to the LGMD2F locus, carrying the E93X mutation described in this report. Thus, LGMD2F is rare in our population.

The index case presented with a severe course. IF and WB analysis showed total absence of α-, γ- and δ-sarcoglycans and severely decrease of β-sarcoglycan in her muscle tissue. This is the only case who had almost total absence of all four sarcoglycans among our LGMD2 patients.

With the inclusion of this mutation, a total of five mutations has now been identified in δ-sarcoglycan gene to date. All patients reported so far presented early with a severe muscular dystrophy and rapid course, and there has been evidence for complete disintegration of the sarcoglycan complex by immunohistochemical analysis. This may reflect a central role of δ-sarcoglycan within the complex, probably in conjunction with β-sarcoglycan [16].

Our case showed a proximal distribution of muscle weakness with relatively equal involvement of the anterior and posterior compartments of the thigh muscles accompanied by calf hypertrophy. This pattern was indistinguishable from that seen in patients with other sarcoglycanopathies. We could not detect any evidence of cardiomyopathy by echogram during our 8 year follow-up.

There are two animal models of δ-sarcoglycan deficiency, both with significant cardiomyopathy, in addition to muscular dystrophy; the BIO 14.6 Syrian hamster [9], and the δ knock-out mouse (Sgcd null) [17]. δ-SG, together with β- and ε-sarcoglycan is a part of the sarcoglycan complex in the coronary artery and other smooth muscle [18]. In the δ knock-out mouse there is some evidence that cardiomyopathy may develop on the basis of coronary artery smooth muscle dysfunction causing ischemic damage to the heart muscle [17]. However, in humans with proven primary δ-sarcoglycan deficiency, severe cardiomyopathy has not been reported yet [19]. There, however, is a LGMD2 subgroup of patients with sarcoglycanopathies in whom a severe cardiomyopathy develops [20]. The molecular basis of the sarcoglycanopathy in these patients has not been completely clarified (although one quite likely has δ-sarcoglycanopathy). A recent observation of reduced coronary reserve in patients with sarcoglycanopathies gives the priority to the vascular component in the development of cardiomyopathy [21], but it remains unclear why this should affect only a subgroup of patients with mutation in one of the smooth muscle sarcoglycans (δ- and β-sarcoglycans). Several hypothesis to explain the underlying reason for this paradox have been put forward; including tissue specific expression, which may affect different arrangements of sarcoglycans [9], and the natural differences between BIO 14.6 Syrian hamster model and humans [22]. Part of the reason may be differences in the actual mutation in the sarcoglycan genes, differences in genetic background or age dependent presentation. In the original article by Coral-Vazquez et al. [17], the Sgcd null mice developed significant cardiomyopathy only after 3 months of age. In addition, treadmill exercise accentuated the cardiac pathology. Our patient, who is wheelchair bound at 16 years, may be too young and immobile to develop cardiomyopathy. Formal assessment of coronary reserve and exercise tolerance could not be performed in this patient. Thus, a high degree of alertness towards the presence of cardiomyopathy remains important, especially in patients with δ- and β-sarcoglycanopathy.

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